Sex-omics: Sex-related Differentially Expressed Genes in the Brain

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Abstract

Life sciences research often does not include both sexes in the design and execution of experiments involving animals and cells. In the USA, the NIH has chosen to address this issue by developing policies to researchers to include both sexes in grant applications. We have data from a transcriptomic analysis of the mouse hippocampus from six inbred strains of mice that provide a novel and deep perspective on the issue of male-female gene expression differences in the brain. We find only 12 genes that are differentially expressed when comparing all males versus all females from the six inbred strains. We have termed these 12 genes the core sex-related differentially expressed genes (DEGs). In contrast, there are >2,000 non-core, sex-related DEGs found when comparing males versus females from the individual strains. The set of the non-core sex-related DEGs differ significantly across the set of inbred strains. This is significant and exciting because the existence of the non-core DEGs provides a basis for a mechanism to explain sex biases in disease susceptibility. As a first test of this hypothesis, we investigated sex-specific patterns of gene expression in a mouse model of Alzheimer's disease which is twice as prevalent in females as males. We expect to find a subset of the non-core, sex-related DEGs to be associated with pathogenic alterations in gene expression.

Materials & Methods

Sample Preparation:

Next generation sequencing libraries were prepared according to Mortazavi et al., 2008 and Chang et al., 2011. Libraries were multiplexed (6 libraries per lane), and 2nM of each multiplexed library were sequenced on an Illumina HiSeq 2500 (Translational Science Laboratory at the College of Medicine, Florida State University). For each condition, three NGS libraries from separate animals (3 biological replicates) were sequenced. We generated approximately 20 million sequencing reads per sample from a 100 base pair sequencing run.

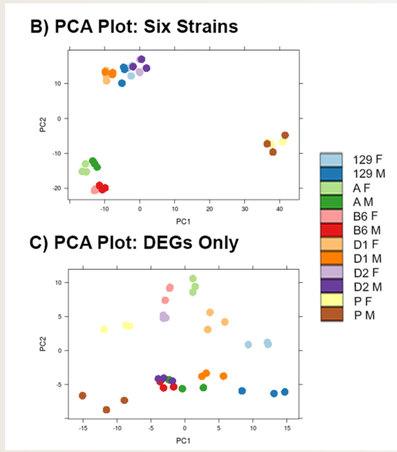
RNA-Seq Data Analysis:

1.QC analysis of each library using fastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/).
2. Adapter and poor quality base trimming with Trimmomatic (Bolger et al., 2014).
3. Align and map sequencing reads with Tophat 2.0.13 (Trapnell et al., 2009) to the mouse genome (current genome release GRCm38) and Bowtie2 2.2.4 (Langmead and Salzberg, 2012).
4. Read counts for each gene are generated by EasyRNASeq (Delhomme, 2012). These counts are used as a measure of abundance and will be used for differential gene expression analysis.
5. DESeq2 (Love et al., 2014) is used to determine statistically significant differentially expressed genes (a False Discovery Rate, FDR, of <0.05 will be used).
6. Functional assessment of genes that are statistically differentially expressed using Gene Ontology (GO) KEGG pathway and phenotype pathway analysis using Webgestalt (Zhang et al., 2005) .

Results

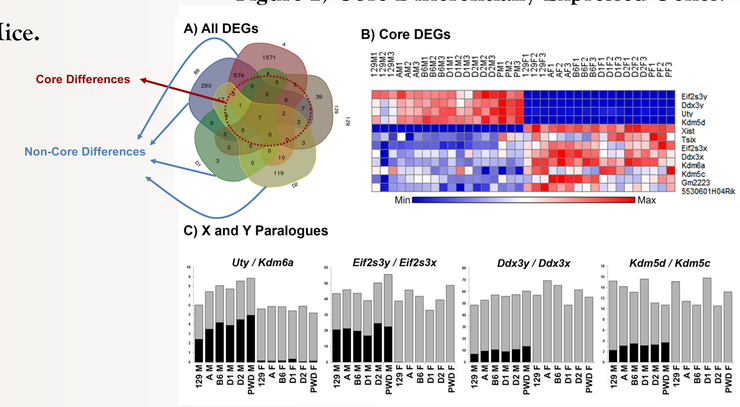
Figure 1) Transcriptomic Analysis of Male and Female Mice from 6 Inbred Strains of Mice.

A)	Male vs Female Transcriptomics		
Strain	Genes Detected	DEGs	Male/Female Upregulated
129	15473	71	49/22
A	15842	2185	1239/946
B6	15810	911	488/423
D1	15604	15	7/8
D2	14951	166	19/147
PWD	14824	10	4/6



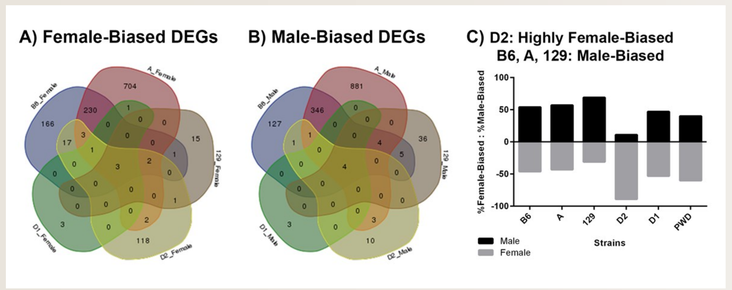
RNA-Seq was performed on mRNA from the hippocampus of 3 males and 3 females from six inbred strains of mice [129S1/SvImJ (129), A/J (A), C57BL/6J (B6), DBA/1J (D1), DBA/2J (D2) and PWD/Ph (PWD)]. A) Tabular representation of the number of genes detected, the number of differentially expressed genes (DEGs) and the direction of the sex-bias of the DEGs. The number of DEGs varies from 10 in PWD to 2185 in the A strain. B) Principal component analysis of all samples from 6 strains demonstrates that male and female samples from each strain cluster together. C) Principal component analysis (PCA1) performed specifically on the sex-specific DEGs from all of the comparisons (2669 different genes). The male and female data are strictly divided along the horizontal axis, with male and female data from each strain in the same vertical plane.

Figure 2) Core Differentially Expressed Genes.



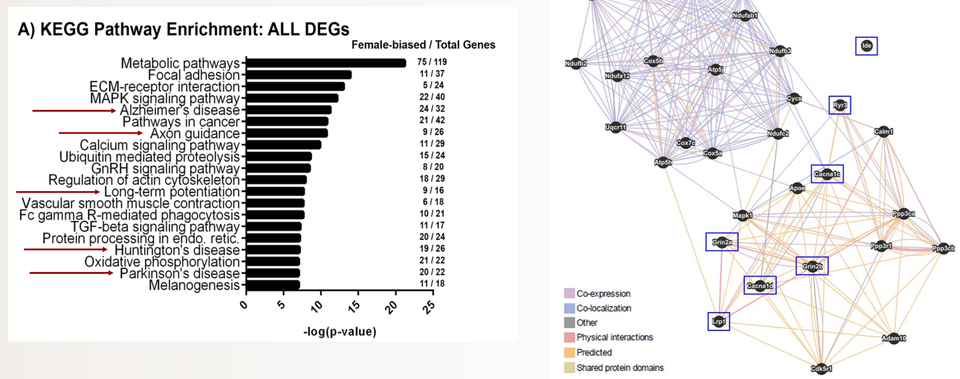
We identified 12 core DEGs that have sex-specific differential gene expression in the hippocampus of males and females. A) Venn diagram of the sex-specific DEGs that overlap between the different strains. The genes that overlap in all strains make up a sub-set of the core DEGs (PWD is not shown due to the constraints of the software; Supplemental Table 2 shows the number and identity of genes that overlap in the six strains). B) Heatmap of the core DEGs in all biological replicates of the 6 strains of mice. Rows represent the gene symbol and columns represent each replicate. The top 4 genes are on the Y chromosome. The minimum to maximum expression is indicated by a gradient from dark blue to dark red, respectively. C) Expression levels of the X and Y paralogues (X – Y genes), which represent 8 of the 12 core DEGs. The average normalized expression for the three biological replicates for each gene pair is plotted. The black and grey part of the bar is the expression of the Y and X chromosome gene, respectively. The x axis indicates the strain and sex and the y axis is the normalized expression value. Note that each graph has a different scale for the y axis.

Figure 3) Sex-biased Differentially Expressed Genes.



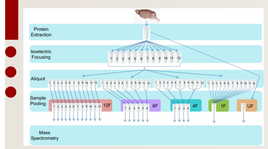
All genes that were differentially expressed in each strain were categorized according to sex-biased expression, genes that are more highly expressed in males or females. A) Venn diagram of the female-biased DEGs in 5 strains (PWD is not shown for A or B due to the constraints of the software; Supplemental Table 2 shows the number and identity of genes that overlap in the six strains). B) Venn diagram of the male-biased DEGs in 5 strains. C) Graphical view of the female- and male-biased expression in the 6 strains. The black and grey part of the bar represents the percentage of the DEGs from each strain that are male-biased or female-biased, respectively. This analysis shows that D2 has a majority of DEGs that are higher in females than in males, with 89% of all DEGs being more highly expressed in females compared to males. 129 is strongly male-biased (69% biased), B6 and A are slightly male-biased (B6: 54%; A: 57%) while D1 and PWD are slightly female-biased (D1: 53% and PWD: 60%).

Figure 4) Alzheimer's Disease Pathway Enrichment.

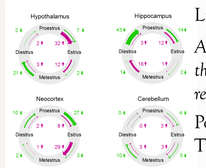


A) KEGG pathway enrichment of DEGs from all 6 strains is represented by a bar graph. The pathways are in order of lowest to highest adjusted p-value, which is represented as the –log of the adjusted p-value on the x axis. The number of genes that are female-biased and the total genes in each category are on the right side of the graph. B) Network of genes that are enriched in Alzheimer's Disease pathway. Two clusters of genes exist with the bottom cluster having fewer connections (lines) than the top cluster. The genes that are male-biased, indicated by blue boxes around gene name, are in the less connected cluster. One of the male-biased genes is isolated with no connections.

Poster Previews



Joseph Bundy will present:
Fractionation-Dependent Improvements in Proteome Resolution in the Mouse Hippocampus by IEF LC-MS/MS
Poster AA12
Tuesday 10/18/15, 2-3pm



Lisa DiCarlo will present:
A transcriptomic analysis of the estrous cycle in four regions on the mouse brain.
Poster 613.14 S9
Tuesday 10/20/15, 2-3pm

Conclusions

- Core Male vs Female differences in all strains of about 20 genes from X and Y chromosomes.
- Non-core Male vs Female differences of 10 to hundreds of genes that vary by strain.
- Male vs Female up-regulation varies across the strains.
- Female bias in expression of Alzheimer's disease is consistent with higher incidence of AD in human females.
- Three genes implicated in Alzheimer's disease that are upregulated in male mice may provide neuroprotection.